ANTIBODIES TO ZIKA VIRUS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/366,782, filed Jul. 26, 2016, the disclosure of which is hereby incorporated by reference in its entirety.

GOVERNMENTAL RIGHTS

[0002] This invention was made with government support under A1077955 and HHSN272201400018C awarded by the NIH. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present disclosure relates to antibodies specific to Zika virus and methods for detecting Zika virus infection in a subject. The present disclosure also relates to therapeutic antibodies useful in reducing viral load.

BACKGROUND OF THE INVENTION

[0004] Zika virus (ZIKV) is a flavivirus of the Flaviviridae family that is transmitted by Aedes species mosquitoes. ZIKV is closely related to the four serotypes of dengue (DENV) as well as other globally relevant viruses including yellow fever (YFV), West Nile (WNV), and Japanese encephalitis (JEV) viruses (Lazear and Diamond, 2016). Since its identification almost 70 years ago, there were few studies of ZIKV until this past year, when large epidemics in the Americas were accompanied by unexpectedly severe clinical manifestations. Although in most instances ZIKV infection results in a mild febrile illness associated with rash and conjunctivitis, severe neurological phenotypes have been described including Guillain-Barré syndrome and meningoencephalitis. Moreover, infection in pregnant women and mice is now linked causally to fetal abnormalities including microcephaly, spontaneous abortion, and intrauterine growth restriction due to placental insufficiency.
[0005] ZIKV infection during pregnancy has emerged as a global public health problem because of its ability to cause severe congenital disease. Thus, there is a need in the art for means to detect and treat Zika virus infection.

BRIEF DESCRIPTION OF THE FIGURES

[0006] The application file contains at least one drawing executed in color. Copies of this patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.
[0007] FIG. 1A, FIG. 1B and FIG. 1C depict flow cytometry plots and graphs showing the profile of neutralizing mAbs against ZIKV. (FIG. 1A) Specificity pattern of mAb reactivity. Cells were infected with DENV-1, DENV-2, DENV-3, DENV-4, or ZIKV (H/PF/2013), harvested, fixed with paraformaldehyde, and permeabilized. Cells were stained with indicated anti-ZIKV mAbs (ZV-2, ZV-13, ZV-48, ZV-54, ZV-64, and ZV-67) or isotype controls and processed by flow cytometry. The data is representative of several independent experiments. (FIG. 1B) Binding to recombinant proteins. The indicated flavivirus proteins (ZIKV E, ZIKV E-FL [fusion loop mutant], ZIKV DIII, WNV E, and DENV-4 E) were purified (see Methods),

adsorbed to 96-well plates, and incubated with the indicated anti-ZIKV MAbs (ZV-2, ZV-13, ZV-48, ZV-54, ZV-64, and ZV-67) or controls (WNV E60 [flavivirus cross-reactive] and WNV E24 [WNV type-specific]. Binding was determined by using an ELISA and the results are representative of two independent experiments performed in triplicate. (FIG. 1C) Neutralization studies. 100 FFU of different ZIKV strains (H/PF/2013, Paraiba 2015, Dakar 41519, and MR-766) were incubated with increasing concentrations of the indicated mAbs in triplicate for 1 h at 37° C. prior to infection of Vero cells. Subsequently, a methylcellulose overlay was added and 40 h later, monolayers were fixed, and stained with 500 ng/ml of ZV-16 (see Methods). Foci were counted and linear regression analysis was performed to generate neutralization curves. The results reflect pooled data from two independent experiments performed in triplicate.
[0008] FIG. 2A, FIG. 2B, FIG. 2C, FIG. 2D and FIG. 2E depict graphs showing differential binding and ADE activity of different anti-ZIKV mAbs. (FIG. 2A, FIG. 2B, FIG. 2C) Quantitative analysis of monovalent DIII binding to anti-ZIKV mAbs by BLI. Shown in the top panel are representative binding curves (sensograms) obtained by passing different concentrations of DIII over biotin-labeled anti-ZIKV antibody immobilized on a streptavidin biosensor surface. The kinetic values were obtained by simultaneously fitting the association and dissociation responses to a 1:1 Langmuir binding model (KD, kinetic). The lower panels show the steady-state analysis results for the same BLI data (KD, equilibrium). Plotted in the lower panels (open circles) is the binding response (nm) versus concentration of DIII offered. In each case the binding was saturable. Lower panel insets, Scatchard plots, suggest a single binding affinity for each interaction. The data is representative of two independent experiments per antibody. (FIG. 2D) Binding of anti-ZIKV mAbs to ZIKV SVPs. (Left) ZIKV SVPs were adsorbed to 96-well plates. After washing and blocking of non-specific binding sites, the indicated biotinylated anti-ZIKV (ZV-2, ZV-13, ZV-48, ZV-54, ZV-64, and ZV-67) or control (WNV E60 [flavivirus cross-reactive] and WNV E16 [WNV type-specific] mAbs were added, and binding was measured by ELISA. (Right) The relative avidity of binding was calculated. The binding curves are representative of five independent experiments, and the avidity values reflect the mean of the five experiments. Error bars indicate standard deviations. (FIG. 2E) ADE studies. Serial dilutions of anti-ZIKV (ZV-2, ZV-13, ZV-48, ZV-54, ZV-64, and ZV-67) or control (WNV E60 [flavivirus cross-reactive] and WNV E16 [WNV type-specific] mAbs were mixed with (left) ZIKV H/PF/2013 or (right) DENV-2 RVPs (which encode for GFP) prior to infection of FcγRIIa$^+$ human K562 cells. Cells were harvested 48 hours after infection and processed by flow cytometry. The data is expressed as the percentage of cells expressing GFP as judged by flow cytometry, and one representative experiment of two is shown. Error bars indicate the range of duplicate technical replicates.
[0009] FIG. 3A, FIG. 3B and FIG. 3C depict structures of anti-ZIKV Fabs and scFv complexed with DIII. (FIG. 3A) Ribbon diagrams of four ZIKV DIII (H/PF/2013) complexes with antibody fragments. The crystal structure of (outer left) ZV-2 Fab (green, elbow angle of 166 degrees), (inner left) ZV-48 scFv (cyan), (inner right) ZV-64 Fab (cyan, elbow angle of 120 degrees), and (outer right) ZV-67 Fab (magenta, elbow angle of 193 degrees) are shown with light